VALIDATION AND USE FOR MARKER-ASSISTED SELECTION OF SCAR MARKER LINKED TO COMMON BEAN RUST RESISTANCE GENE *UR-5*

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Conventional breeding methods have been widely used to develop new common bean (*Phaseolus vulgaris* L.) cultivars resistant to rust disease. However, this resistance can be easily overcome due to the extensive virulence diversity of the rust fungus *Uromyces appendiculatus* (Pers.: Pers) Unger. Resistance gene pyramiding assisted by molecular markers has been proposed as an alternative strategy to overcome this problem. DNA markers tightly linked to the resistance genes may be used for the indirect selection of resistant plants in segregating populations, without the need for multiple inoculations.

In Brazil, Faleiro *et al.* (1999) demonstrated that the Middle American differential cultivar Mexico 309 (gene *Ur-5*) is immune to nine and moderately resistant to two of 13 *U. appendiculatus* pathotypes identified in the state of Minas Gerais. The rust resistance gene *Ur-5* is also effective against 11 *U. appendiculatus* pathotypes from Goiás state (Santos & Rios, 2000). These data indicate that 'Mexico 309' is an important rust resistant source for Central Brazil. Haley *et al.* (1993) reported the identification of $OPI19_{460}$ RAPD (Random Amplified Polymorphic DNA) molecular marker as linked in coupling phase and without recombinants to *Ur-5*. This RAPD marker was converted to the SCAR (Sequence Characterized Amplified Region) marker $SI19_{460}$ by Melotto & Kelly (1998).

Data reported by Alzate-Marin *et al.* (2004) indicate that *U. appendiculatus* resistance of 'Mexico 309' is controlled by a single dominant gene which is distinct from genes *Ur-11* (cv. PI 181996 and line Belmidak RR-3) and *Ur-ON* (cv. Ouro Negro). The main goal of this work was to evaluate if the molecular marker $SI19_{460}$ would be useful in BIOAGRO/UFV breeding program, which involves crosses between "carioca-type" cultivar Rudá, a Brazilian commercial cultivar susceptible to rust, and 'Mexico 309'. In this program the rust resistance genes *Ur-5*, *Ur-11* and *Ur-ON* are being used for gene pyramiding in "carioca-type" backgrounds.

One of the primary leaves from 61 BC_3F_2 plants derivates from crosses between cultivars Mexico 309 and Rudá were collected and kept at -80°C for DNA extraction, which was based on Doyle & Doyle (1990). The remaining primary leaves were inoculated with *U. appendiculatus* pathotype 10 (race 29-3). The leaves were scored visually for the rust disease symptoms using a 1-to-6 scale proposed by Stavely *et al.* (1983). Five resistance and susceptible BC_3F_2 individuals were used to construct two contrasting bulks, which were tested with marker $SI19_{460}$. The PCR reactions (25 µL) contained 30 ng of genomic DNA, 0.2 mM of each SCAR primer (F: 5' – AAT GCG GGA GTT CAA TAG AAA AAC C – 3' and R: 5' – AAT GCG GGA GAT ATT AAA AGG AAA G – 3'), 10mM/50mM Tris/KCl (pH 8.0), 2 mM $MgCl_2$, 0.48 mM of total dNTP, and 1 U of *Taq* DNA polymerase. The amplification program included an initial step of 3 min at 94°C; 34 cycles of 94°C/1 min, 50°C/1 min and 30 s, and 72°C/1 min and 30 s; and one final step at 72°C for 7 min.

The bulk analysis (Figure 1) indicated that SCAR marker was indeed linked to the *Ur-5* resistance gene. The analysis of all BC_3F_2 plants confirmed that $SI19_{460}$ marker is linked in coupling phase at 3.31 cM of the gene *Ur-5* in this population (Table 1). The calculated LOD score was 11.0, and the estimated selection efficiency was 91.32%. In addition, $SI19_{460}$ was polymorphic between 'Mexico 309' and the rust resistance sources 'Belmidak RR-3' (gene *Ur-11*) and 'Ouro Negro' (gene *Ur-ON*) (Figure 1).

This SCAR marker is now being used in the molecular marker-assisted pyramiding program at the BIOAGRO/UFV to aid the development of new common bean cultivars with wide and durable resistant to rust and adapted to Central Brazil.

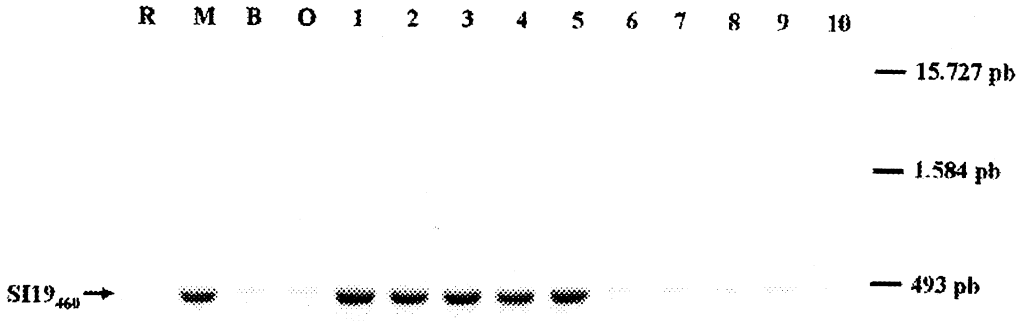

Figure 1. Electrophoretic analysis of amplification products obtained with $SI19_{460}$ SCAR molecular marker. Lanes are as follows: R, 'Rudá' (susceptible); M, 'Mexico 309' (*Ur-5*); B, 'Belmidak RR-3' (*Ur-11*); O, 'Ouro Negro' (*Ur-ON*); 1-5, BC_3F_2 Rudá x Mexico 309 resistant plants; and 6-10, BC_3F_2 susceptible plants to *U. appendiculatus* pathotype 10 (race 29-3). The arrow indicates marker $SI19_{460}$, a DNA band with 460 bp linked in coupling phase to the rust resistance gene *Ur-5* present in common bean cultivar Mexico 309.

Table 1. Segregation for resistance and linkage analysis of molecular marker $SI19_{460}$ and rust resistance gene *Ur-5* in BC_3F_2 population derived from a crosses between the cultivars Rudá and Mexico 309 (*Ur-5*)

Locus tested	Expected ratio	Observed ratio[a]	χ^2	P(%)[b]	cM[c]
Ur-5	3:1	44(R):17(S)	0.2677	60.48	-
$SI19_{460}$	3:1	46(+):15 (-)	0.0054	94.11	-
Ur-5/$SI19_{460}$	9:3:3:1	44(R/+):0(R/-):2(S/+):15(S/-)	54.7887	0.00	3.31

[a]Resistant plants (R), susceptible plants (S), presence of marker (+), absence of marker (-).
[b]Probability in percentage.
[c]Genetic distance in centimorgans (cM) of the marker $SI19_{460}$ in relation to resistance gene *Ur-5* with a LOD score of 11.0.

Acknowledgement: This work was supported by grants from CNPq and FAPEMIG (Brazilian Government). T. L. P. O. de Souza was supported by an M.S. scholarship from CNPq.

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